



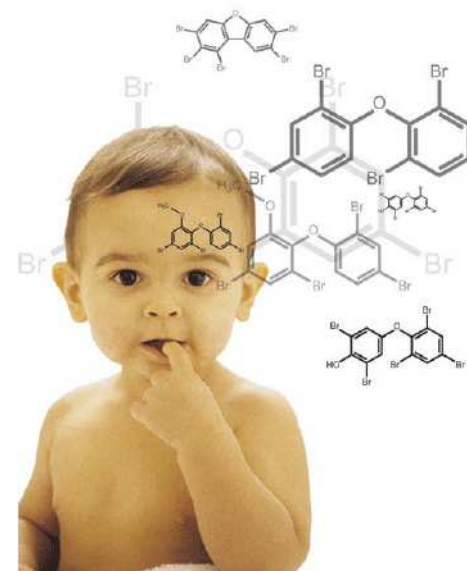
Children's Exposure to Flame Retardant Chemicals (Old and New) in Indoor Environments

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Outline

1. Introduction and Background

- a. What is a flame retardant (FR) and how do they work?
- b. What regulations govern the use of FRs in products?
- c. What type of products contain FRs?
- d. What type of FRs are used in consumer products?

2. Early Exposure to PBDEs

- a. **Serum PBDEs in a U.S. Pregnancy Cohort: Associations with Thyroid Hormones and Birth Outcomes (Stapleton et al. 2011; Miranda et al. In review)**
- b. U.S. Toddlers Exposure to PBDEs in Indoor Environments: Exposure Pathways and Associations with SES

3. Effects of PBDE Exposure on Thyroid Hormone Regulation

- a. Toxic Mechanisms reported from *in vitro* and animal studies
- b. Human health studies

4. PBDE Replacement Chemicals: Are they Better or Worse?????

- a. Levels of Alt FRs in Indoor Dust
- b. Prenatal Exposure to FM 550 in Rats: Signs of Endocrine Disruption?



Definition:

“A substance added or a treatment applied to a material in order to suppress, significantly reduce or delay the combustion of the material” *EHC:192, WHO 1997*

Regulations That Govern the Use of FRs

U.S. Residential Furniture:

- California Technical Bulletin 117

Electronics:

- Underwriters Laboratory Certifications for Insurance purposes (e.g. UL 746 and -94 V-2 – E&E)

Textiles:

- Children’s Sleepwear (CPSC)
- Seats and Drapes in Public Buildings (NFPA 701, CA TB 133)





What Type of Products are Treated with Flame Retardants in Your Home?



Sleep Positioners









Nursing Pillow

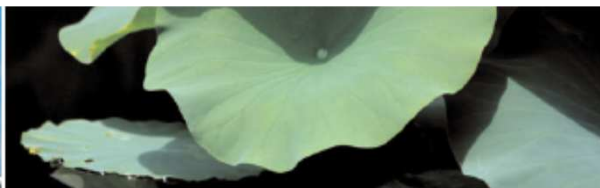




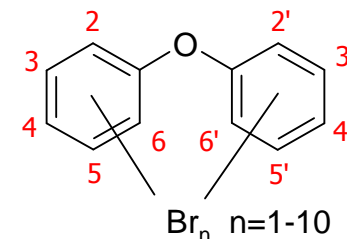
PBDE Commercial Mixtures

Congener (# of Br atoms)	% of Mixture	Product Applications
PentaBDE Commercial Mixture (DE-71; Phased out 2004)		
BDE 47 (4)	38.2	  
BDE 85 (5)	2.96	
BDE 99 (5)	48.6	
BDE 100 (5)	13.1	
BDE 153 (6)	5.44	
BDE 154 (6)	4.54	
OctaBDE Commercial Mixture (DE-79; Phased out 2004)		
BDE 153 (6)	8.66	 
BDE 154 (6)	2.68	
BDE 183 (7)	42.0	
BDE 196 (8)	10.5	
BDE 197 (8)	22.2	
BDE 207 (9)	11.5	
DecaBDE Commercial Mixture (Saytex 102E)		
BDE 206 (9)	2.19	
BDE 207 (9)	0.24	
BDE 208 (9)	0.06	
BDE 209 (10)	96.8	

(La Guardia et al 2006)

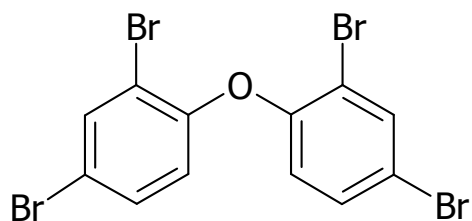


PBDE Nomenclature



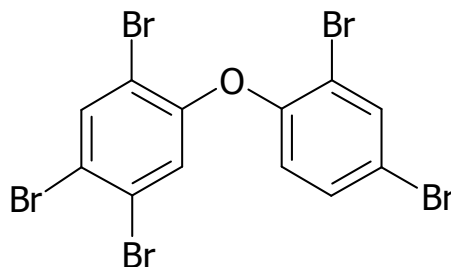
Polybrominated Diphenyl Ether (PBDE)

PentaBDE Congeners:



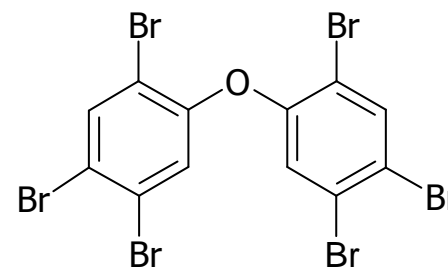
2,2',4,4'-tetrabromodiphenyl ether

BDE-47



2,2',4,4',5-pentabromodiphenyl ether

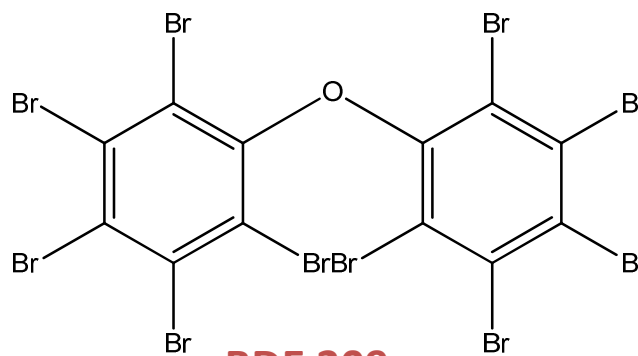
BDE-99



2,2',4,4',5,5'-hexabromodiphenyl ether

BDE-153

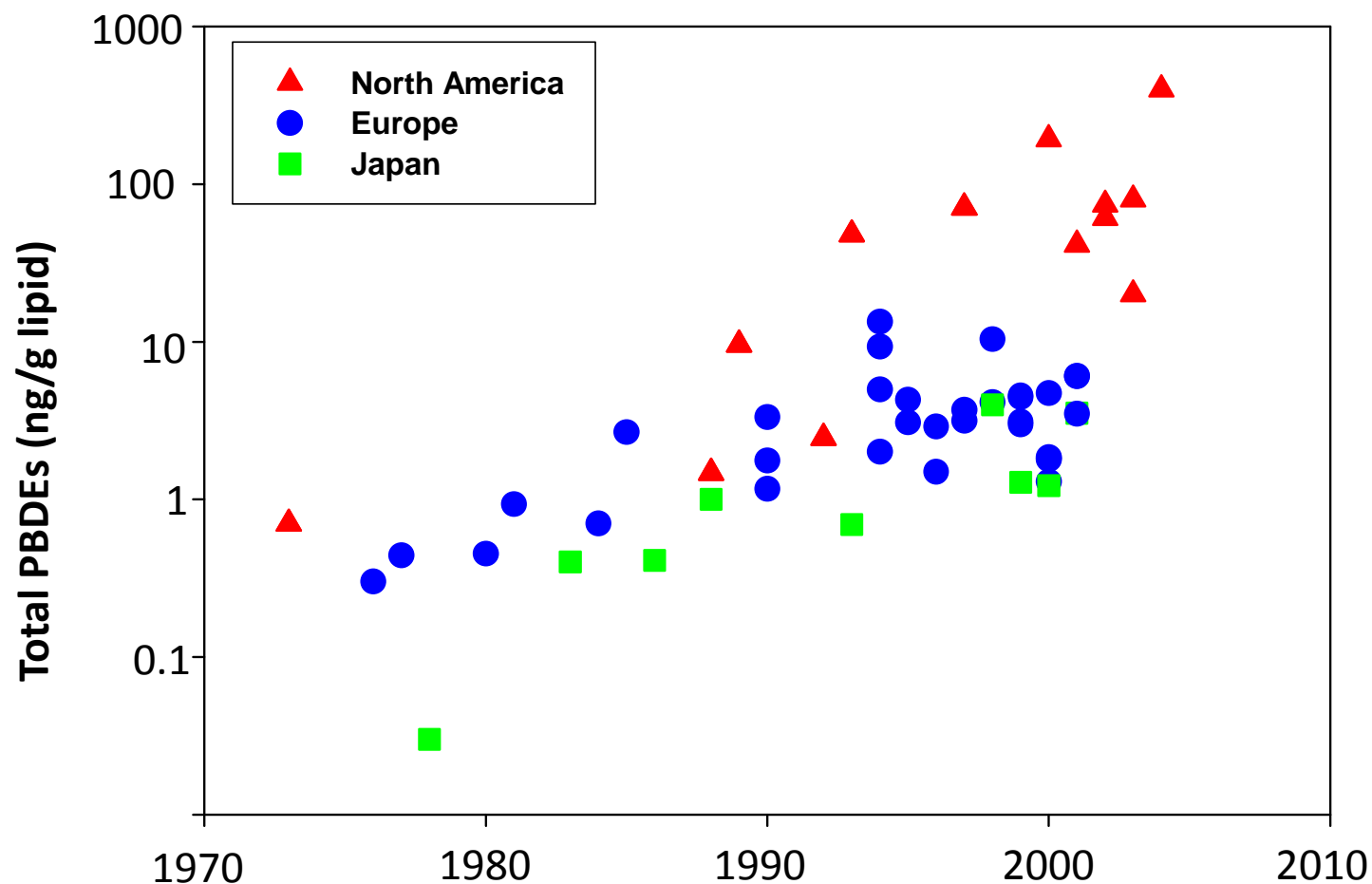
DecaBDE:



BDE 209



PBDEs in Human Samples From Around the World



From Hites et al., 2005



Previous Studies on PBDE Exposure

- Exposure models suggested that *infants* would be receiving the highest exposure due to breast milk ingestion (Jones-Otazo et al., 2005; Schecter et al. 2003)
- Exposure studies in US *adults* have observed significant associations with both diet (Wu et al., 2007; Fraser et al., 2010) and dust (Johnson et al., 2010)
- Fewer studies on children's exposure:
 - Rose et al. (2010) reported levels in 2-5 year old children in California and found concentrations 2-50X higher than adults
 - Windham et al. (2010) measured PBDEs in 6 to 8 year old girls from California and Ohio; significantly higher concentrations in CA vs Ohio; higher in blacks compared to whites
- Quiros-Alcala et al. (2011) measured PBDEs in dust from low-income households; concentrations were among highest measured
- Zota et al. (2010) wrote perspective article on PBDEs and socio-economic disparities



Children's Exposure to Flame Retardants



- Children are spending more time indoors
- Indoor environments are often more polluted than outdoor environments (PBDEs in Dust>>>>>PBDEs in Soils)
- Children have a high number of hand-to-mouth contacts
- Children are physically in contact with many FR treated products



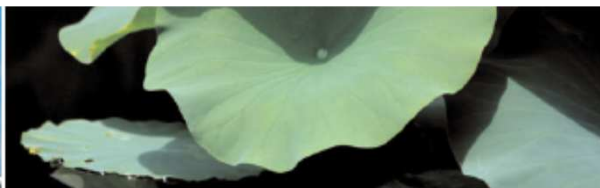


Serum PBDEs in US Toddlers: Associations with Hand Wipes, House Dust and Socioeconomic Variables

(Stapleton et al., 2012; Environmental Health Perspectives)

Research Questions:

- 1. How much exposure are children receiving from exposure to indoor dust?**
- 2. Can we predict exposure by measuring residues on children's hands?**
- 3. Are PBDE exposures higher in families of lower socio-economic status (SES)?**



Methods

Recruitment:

- Targeted families with children between the ages of 12 – 36 months; residents residing in central North Carolina (USA);
- Recruited at the North Roxboro Duke Pediatrics Health Clinic, or by letters;
- Recruited Between May 2009 – September 2010
- All families signed informed consent

Sample Collection:

- Blood sample (venipuncture)
- Hand wipe sample (Investigator Collected)
- House dust sample (Investigator Collected)
- Researcher administered questionnaire



Sample Analysis:

- Serum analyzed for PBDEs (CDC)
- Hand wipes and house dust analyzed for PBDEs and new flame retardants in our laboratory using mass spectrometry





Toddler Cohort Characteristics (N=83)

Characteristic	Number	%
Gender		
Female	42	51
Male	41	49
Age		
12-18 mo	26	31
19-24 mo	34	41
25-36 mo	23	28
Race/Ethnicity		
Hispanic	16	19
Non-Hispanic Black	19	23
Non-Hispanic White	43	52
Other	5	6
Marital Status of Parents		
Married	55	66

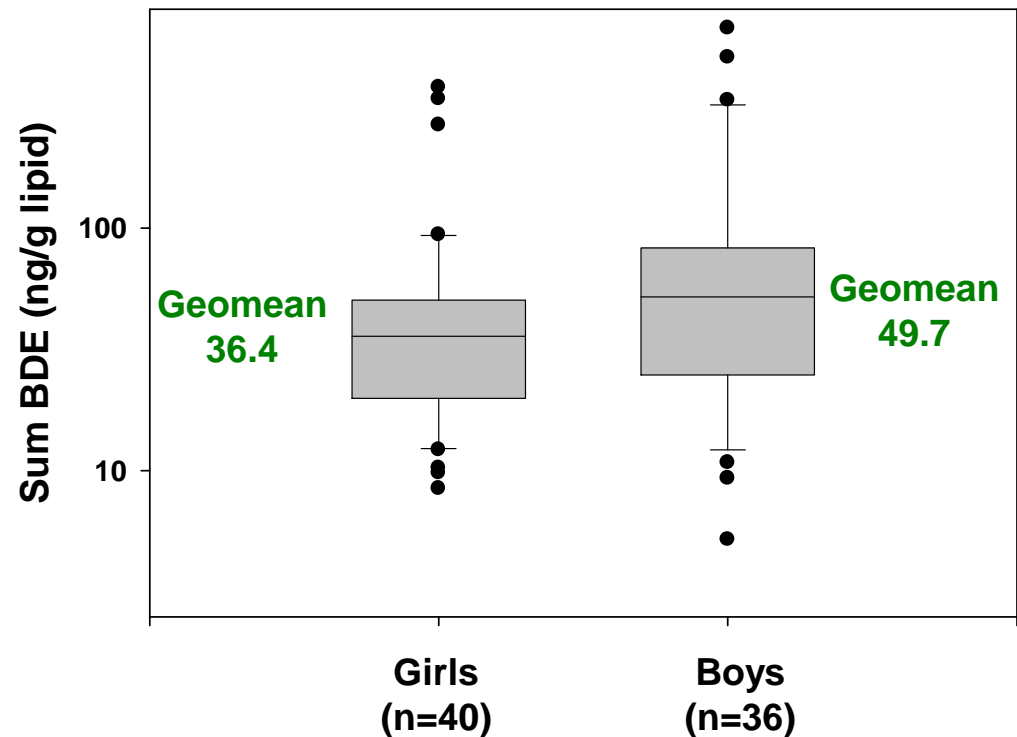
Characteristic	Number	%
Mother's Education Level		
Some High School	8	10
Completed High School	8	10
Some College	12	14
Completed College	25	30
Graduate Degree	30	36
Father's Education Level *		
Some High School	10	14
Completed High School	11	15
Some College	7	9
Completed College	18	24
Graduate Degree	28	38
Breast Feeding Duration		
Less than 1 month	20	27
1 to 6 months	21	28
6 months or more	34	45

* N=73 (Information on Father's Education Level was not reported or unavailable in a few cases).



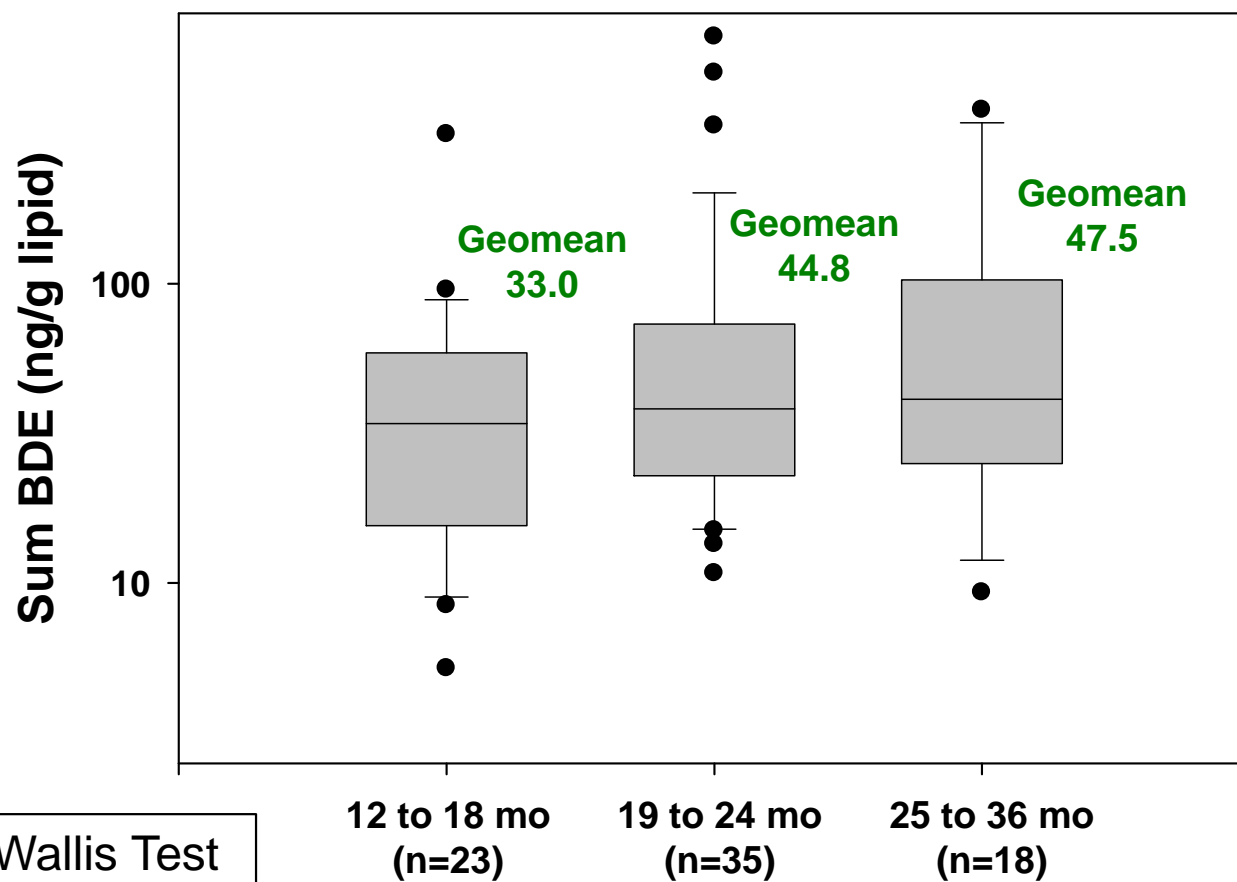
A Comparison of Serum PBDEs by Gender

- Σ BDEs higher in boys relative to girls
- Σ BDE not statistically significant but suggestive ($p=0.086$)
- BDE 100 was significantly different between groups ($p<0.05$)





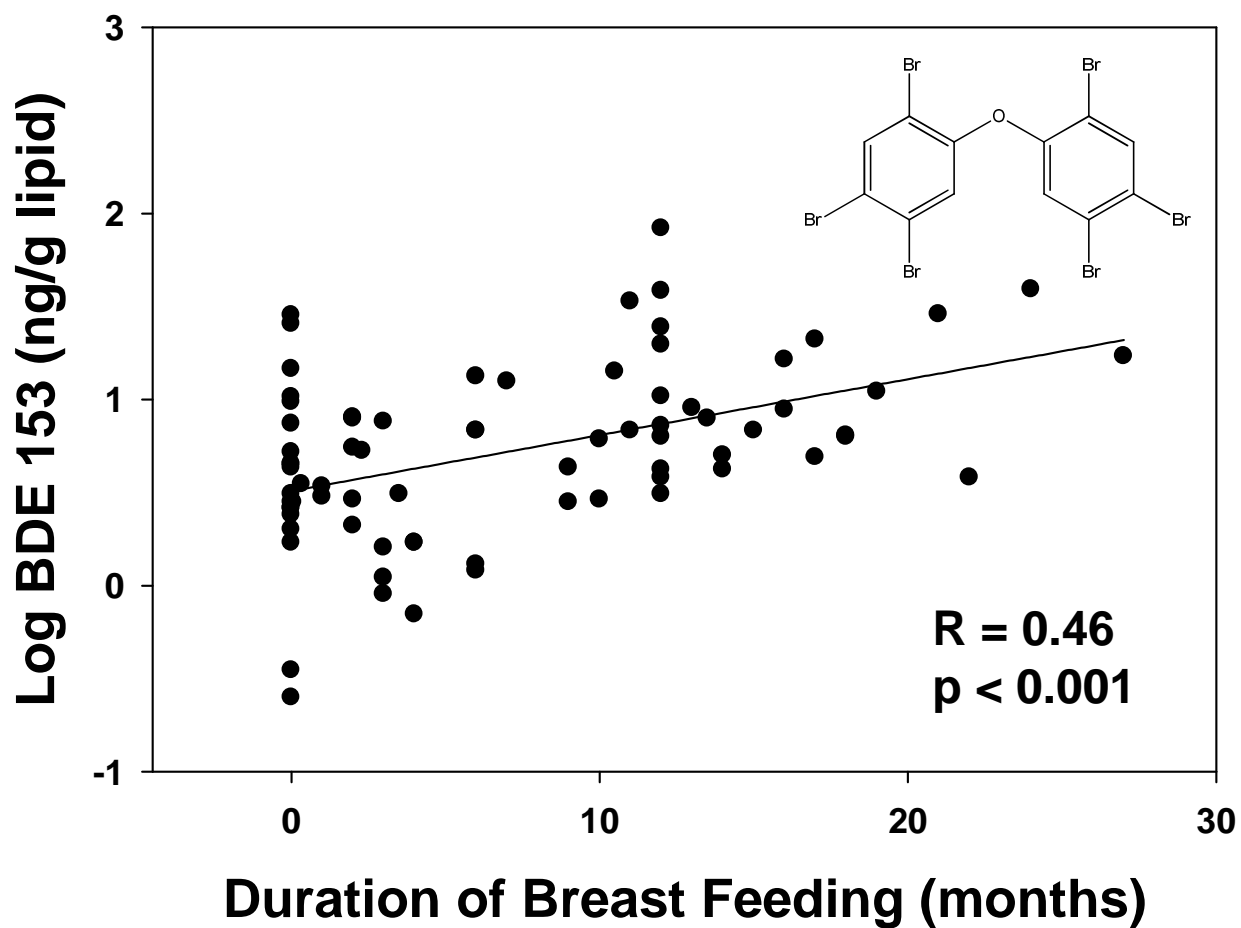
A Comparison of Serum PBDEs by Age



Kruskal-Wallis Test
 Σ BDE, $p=0.59$;
BDE 153, $p<0.05$



The Influence of Breastfeeding on Serum PBDEs



*No trend observed for BDE 47, 99, 100 or Σ BDE



Potential Dust Exposure Paradigm

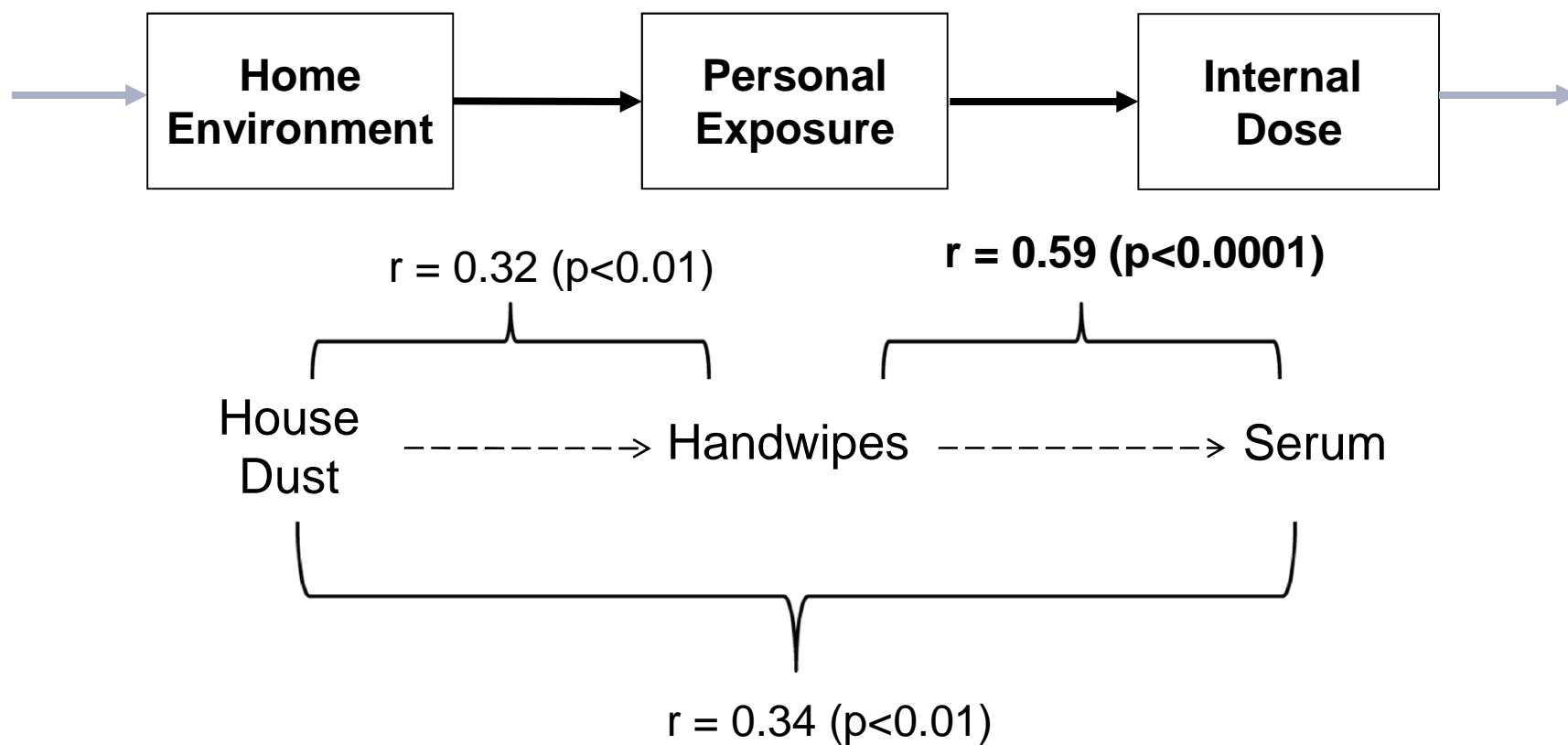


House Dust -----> Handwipes -----> Serum

What are the associations between these three “compartments”?

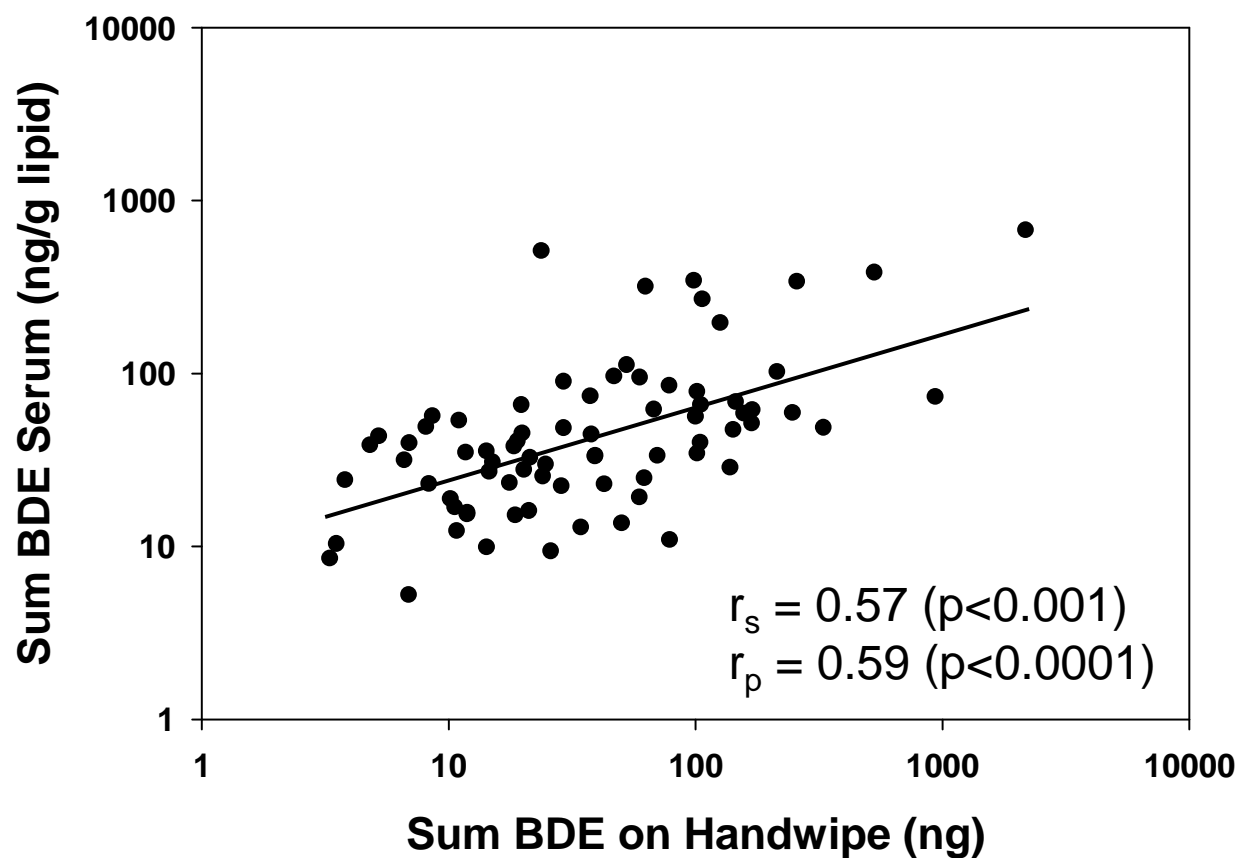


Potential PBDE Exposure Paradigm





PBDE Residues on Your Hands Are Related to What's in Your Body

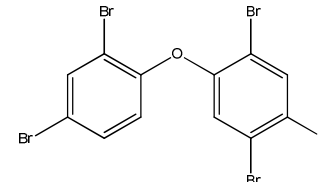
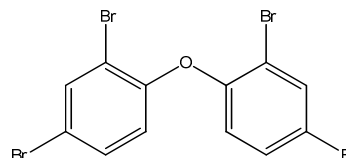




Predictors of Serum PBDE: A Multivariate Analysis

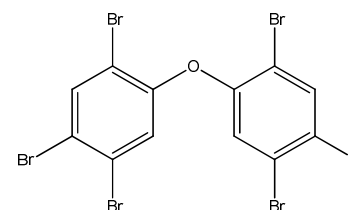
- Σ BDEs 47,99,100:

- Age (60% increase/year; $p=0.05$)
- Paternal Education (2X higher when father had less than college degree; $p<0.01$); race displayed same trend and both variables were highly correlated;
- Handwipe levels (3.66X higher in third tertile compared to lowest tertile; $p<0.01$)



- BDE 153:

- Age (70% increase/year; $p<0.05$)
- Breastfeeding Duration (7%/month; $p<0.001$)
- Handwipe Levels (2.44X higher in third tertile compared to lowest; $p<0.01$)



* Education was categorized by either having a completed college degree (AD, BD, GD) or less than.

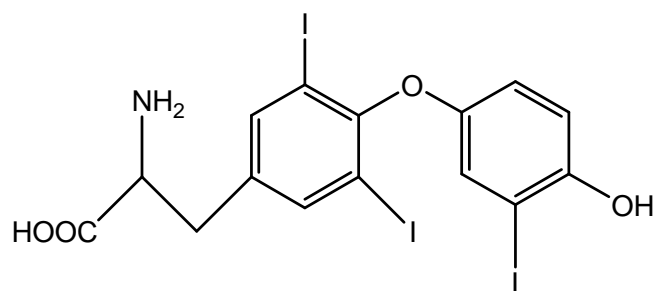


***What are the consequences of this early
life exposure??***

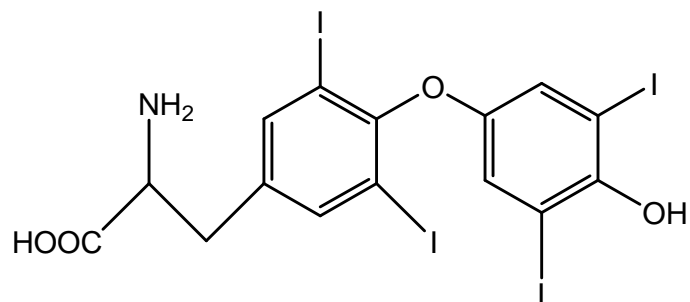


PBDEs are Thyroid Hormone Mimics

Thyroid Hormones

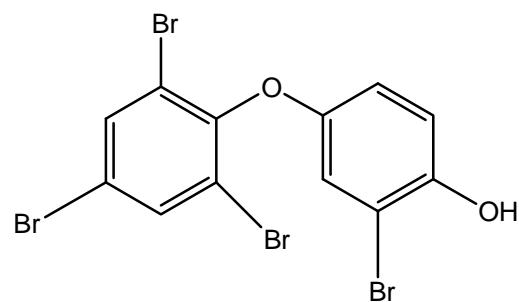


Triiodothyronine (T3)

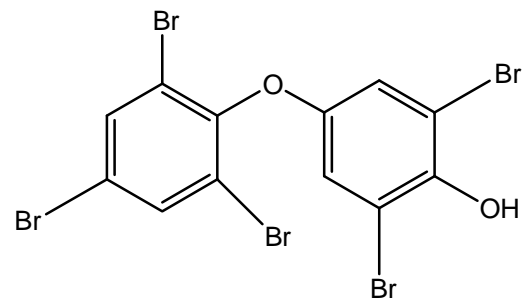


Thyroxine (T4)

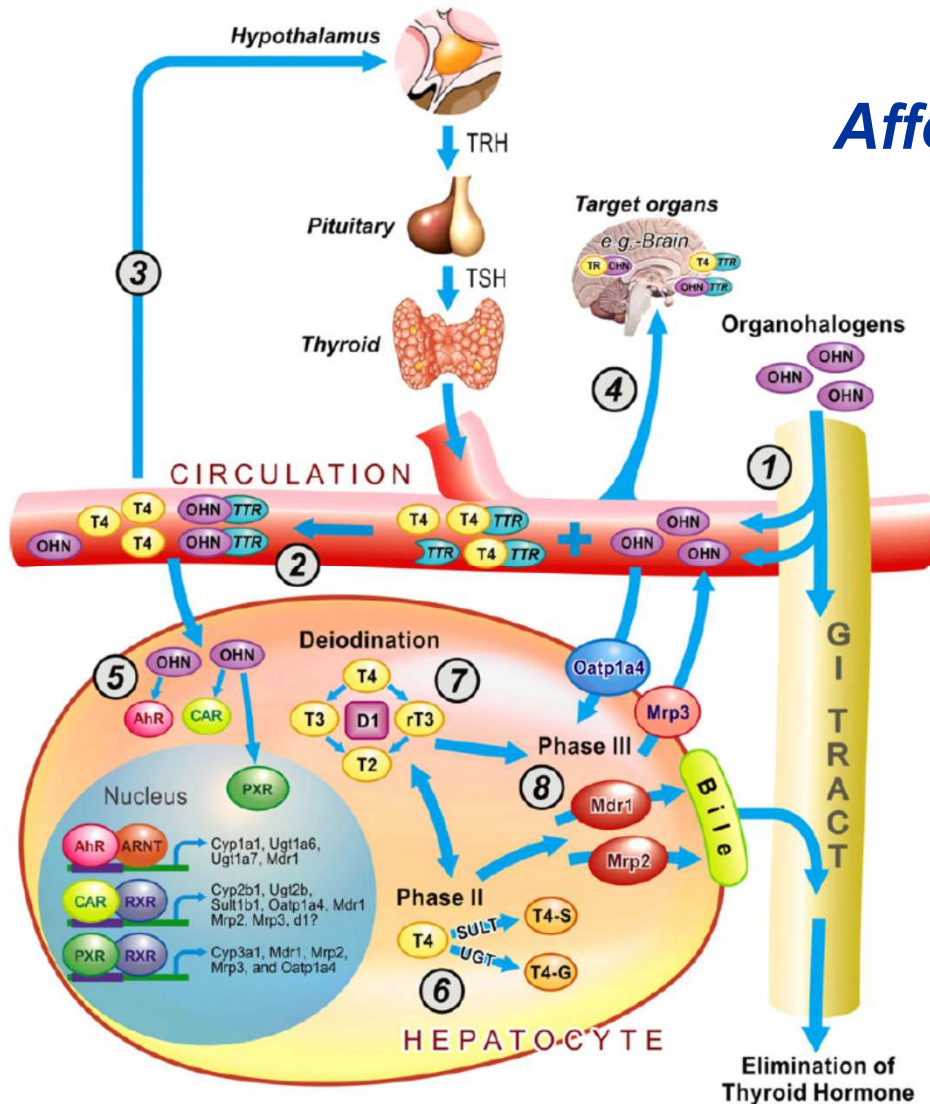
PBDE Oxidative Metabolites



T3-like OH-BDE



T4-like OH-BDE



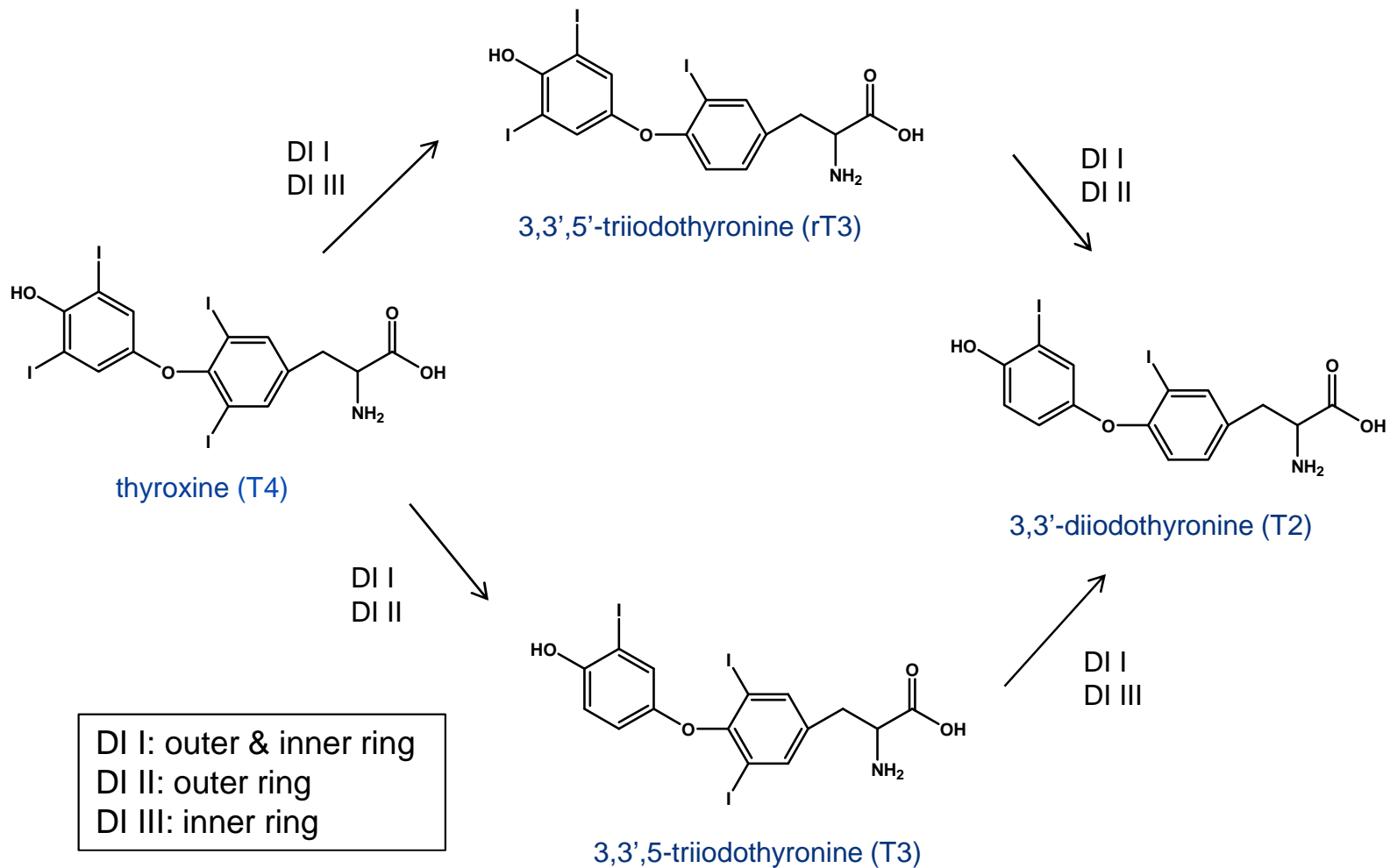
Toxic Modes of Action Affecting Thyroid Regulation

2. PBDE metabolites displace T4 from serum transporters (Meerts et al., 2000);
4. Transporters deliver PBDEs or metabolites to brain where agonism/antagonism with nuclear receptors may occur;
5. Upregulation of xenobiotic metabolizing enzymes (XMEs) (Szabo et al 2009)
6. XMEs conjugate T4; increased or decreased clearance of THs (Butt et al., in Progress);
7. Disruption of Deiodinase Activity by PBDE metabolites (Butt et al., 2011)

(From Kodavanti and Curras-Collazo 2010)

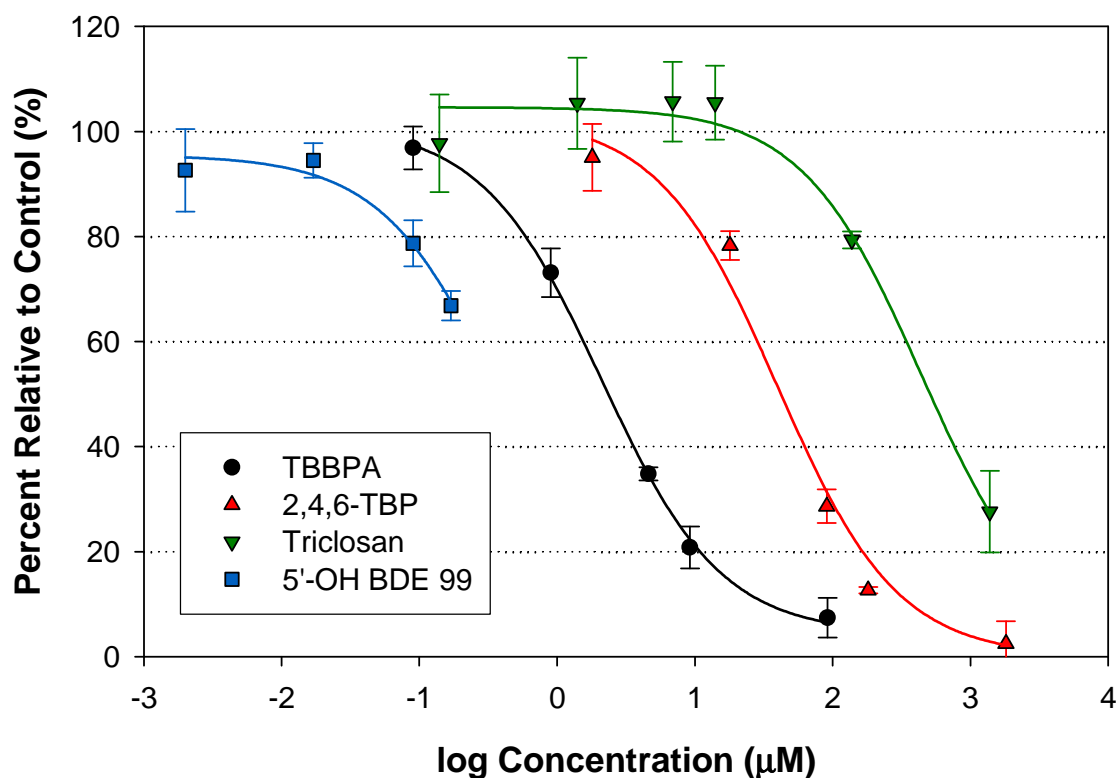


Deiodinase Reactions

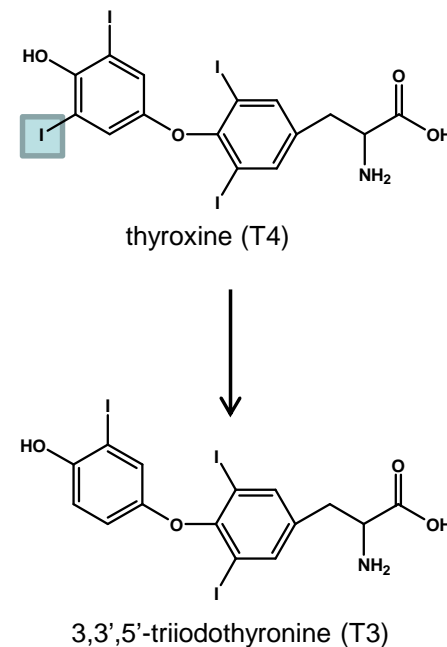




Inhibition of Thyroxine Deiodination by Flame Retardants (Butt et al., 2011)



Mean ± 1 std. deviation (n=3)



In Vitro Experiments Conducted with Pooled Human Liver Microsomal Samples



Neurodevelopmental Effects Observed in Animal Studies

- PBDEs shown to affect development of fetal human neural progenitor cells *in vitro* which was mediated by thyroid hormone signaling (Schreiber et al. 2010)
- Studies conducted in rodent models observed significant alterations in spontaneous behavior and habituation, deficits in learning and memory, and changes in cholinergic nicotinic receptors, primarily occurring when exposure occurs during “rapid brain growth” (Eriksson et al., 2001,2002; Viberg et al., 2003, 2006, 2007).
- Mice exposed to BDE 209 during rapid brain growth were observed to have altered expression of CAMKII, GAP-43 and BDNF in different regions of the brain (Viberg et al., 2007).



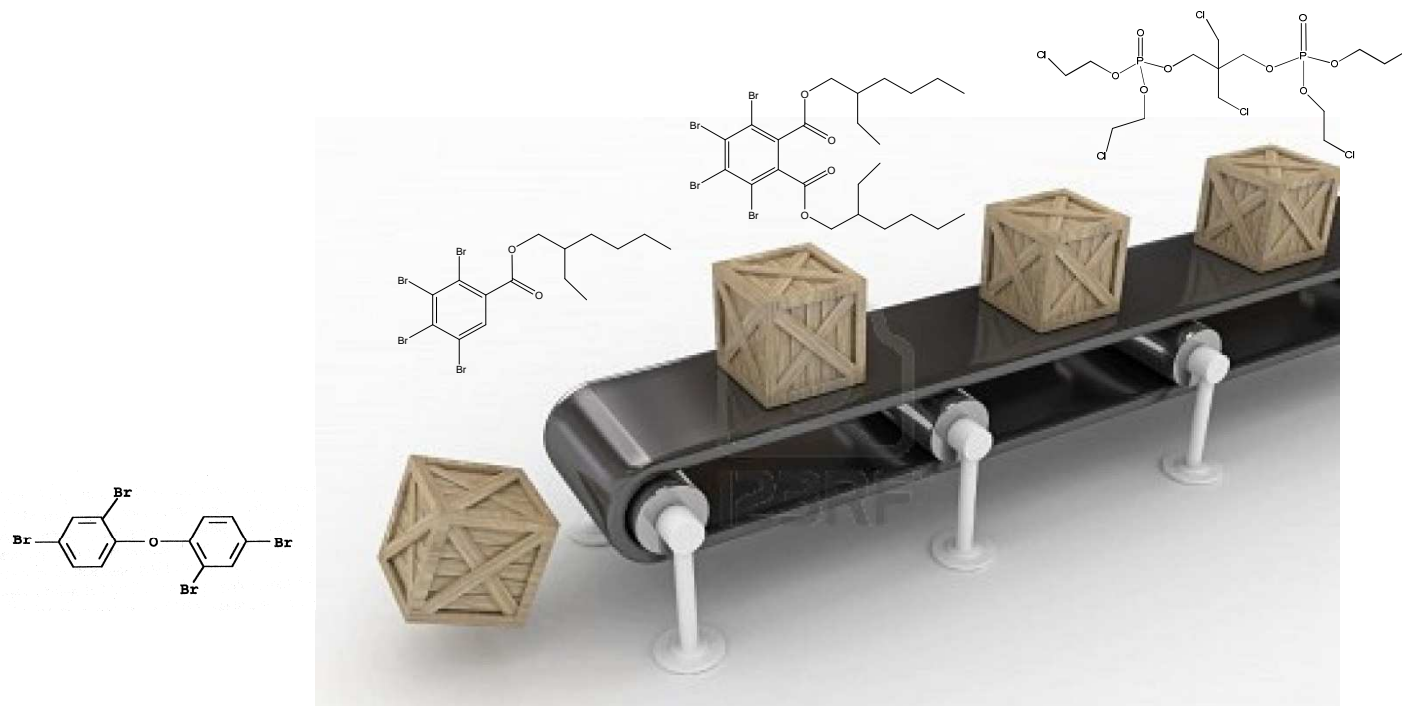
Neurodevelopmental Deficits Associated with PBDEs in Children *(Herbstman et al. 2010)*

- PBDE levels in cord blood at birth were negatively associated with:
 - Mental Developmental Index at 24 months of age (BDEs 47, 99, and 100, univariate and adjusted models);
 - Full and Verbal IQ at 48 months (BDE 47 and 100, adjusted models);
 - Full and Performance IQ at 72 months (BDE 100 and 153; univariate and adjusted models)



The Case of the Chemical Conveyor Belt.....

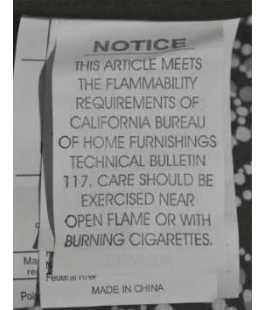
When one flame retardant is banned, another chemical moves in to take its place, and less is known about the replacement chemical...





Flame Retardants in Consumer Products

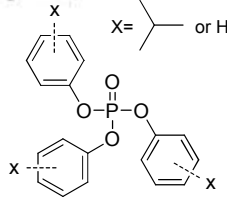
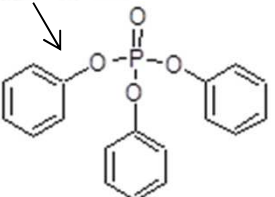
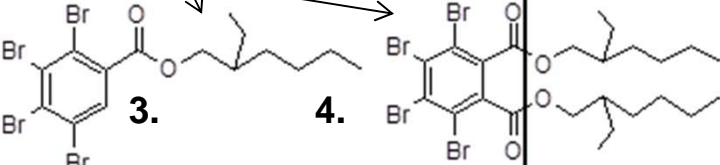
- Identification of FRs in Baby Products (*Stapleton et al 2011*)
 - Screened 102 baby products (car seats, nursing pillows, sleep positioners, etc)
 - 80% contained FR
 - “Chlor Tris”, and FM 550 most common FRs identified
- Identification of FRs in Residential Furniture (*Stapleton et al. In Draft*)
 - Screened 101 different samples from couches
 - 86% contained FR
 - “Chlor Tris”, and FM 550 most common FRs identified





Firemaster® 550 – 4 Components

(Stapleton et al. 2008)

SECTION II - COMPOSITION/INFORMATION ON INGREDIENTS			
INGREDIENT NAME	CAS No.	%	EXPOSURE LIMITS
*Component B Triaryl phosphate, isopropylated 	TS TS	40 - 60 24 - 51	Y (Hazardous) Not established (OSHA PEL TWA) Not established (OSHA PEL STEL) Not established (OSHA PEL CEIL) Not established (ACGIH TLV TWA) Not established (ACGIH TLV STEL) Not established (ACGIH TLV CEIL)
2. Triphenyl phosphate 	115866	6 - 24	N (Hazardous) 3 mg/m3 (OSHA PEL TWA) Not established (OSHA PEL STEL) Not established (OSHA PEL CEIL) 3 mg/m3, A4 (ACGIH TLV TWA) Not established (ACGIH TLV STEL) Not established (ACGIH TLV CEIL)
Component A 	TS	40 - 60	Y (Hazardous) Not established (OSHA PEL TWA) Not established (OSHA PEL STEL) Not established (OSHA PEL CEIL) Not established (ACGIH TLV TWA) Not established (ACGIH TLV STEL) Not established (ACGIH TLV CEIL)

*Indented chemicals are components of previous ingredient.

Additional Information

Additional exposure limits for triphenyl phosphate:
NIOSH REL TLV = 3 mg/m3



EPA Issues Consent Order on FM 550

- EPA required further testing on FM 550 components
- **Oral two generation reproduction and fertility study in rats** – Observed significant effects on body weight in dams and pups; F2 pups had significant effects on reproductive traits and thyroid at 50 mg/kg/day
 - **No Observable Adverse Effect Level (NOAEL) = 50 mg/kg/day**
- **Prenatal developmental toxicity in rats** – Observed significant effects on body weight in dams and pups: pups also displayed fused cervical vertebral neural arches and incomplete ossification of skull bones at 100 and 300 mg/kg/day
 - **NOAEL = 50 mg/kg/day**
- **Migration Study from foam** simulating wet transfer of the mixture to skin via dermal contact with upholstery. Data demonstrated that the substance is not absorbed via dermal contact with foam.



FM550 Reproductive and Development Study

(Collaboration with Dr. Heather Patisaul, NC State)

- Pregnant Wistar Rats (~350 g) exposed from GD6 to PND 21
- Exposed to 20 uL of each treatment on cookie once per day; water and food ad libitum
- Treatment Groups (n=3 dams/treatment):

A – Control

B – High Dose (3.01 mg/kg/day)

C – Low Dose (301 ug/kg/day)

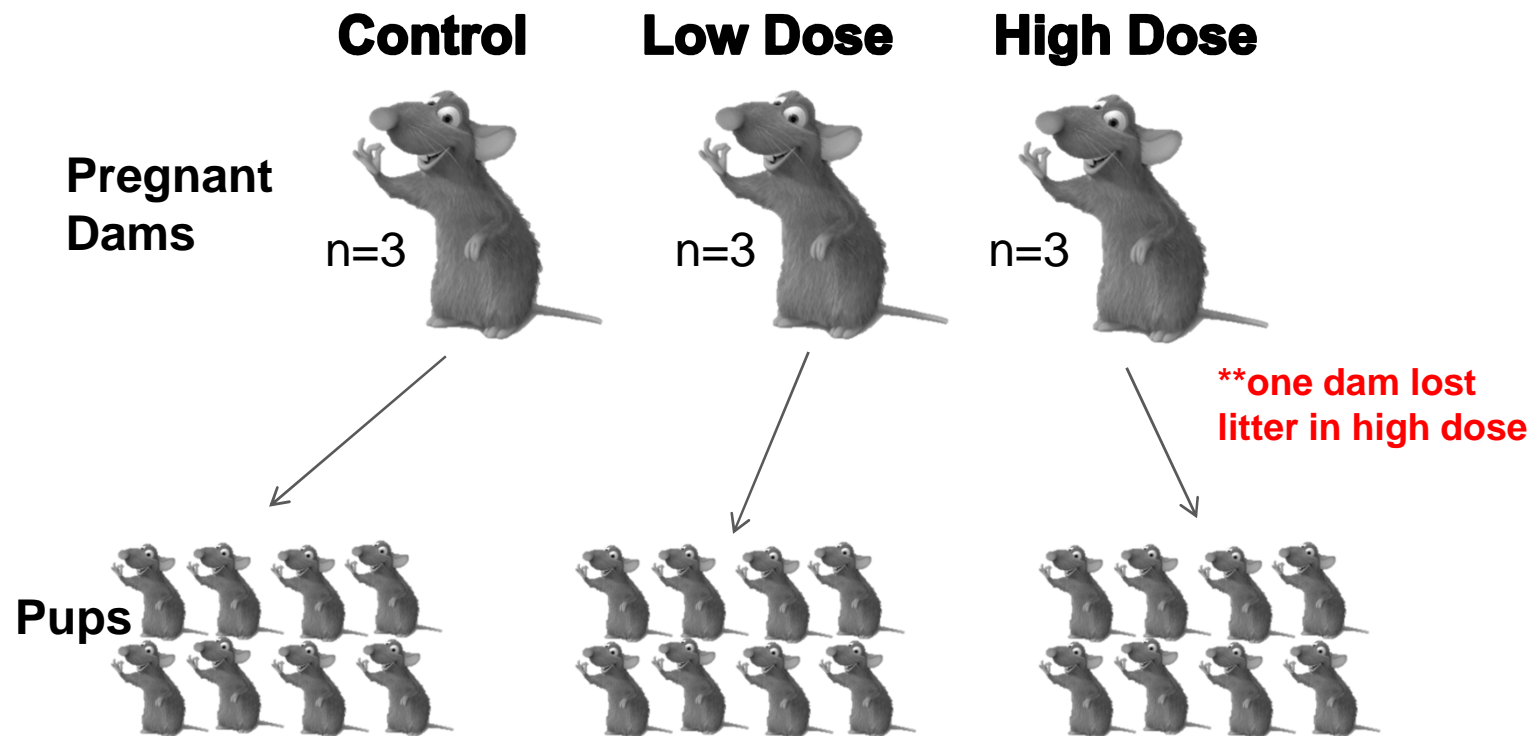
- Monitor uptake of TBB and TBPH in dams/pups
- Examine effects on pup growth, thyroid levels, reproductive development and behavior





FM550 Rat Exposure Design

Dams: collected serum, liver, brain, fat, and muscle on PND 21

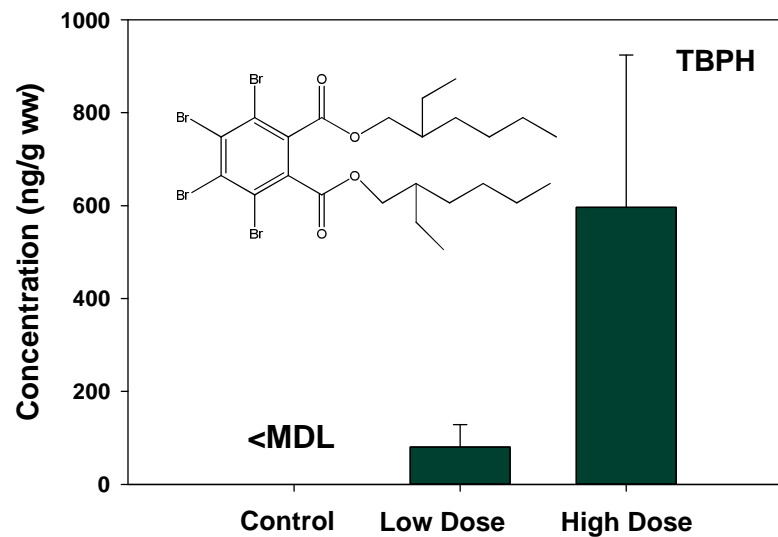
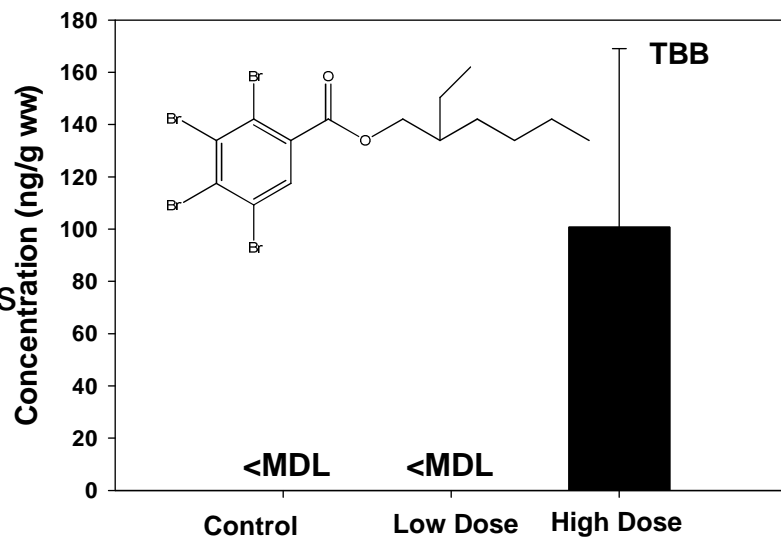


Pups: collected serum (limited), liver, brain, fat, and muscle from pups on PND 21, and 7 months of age

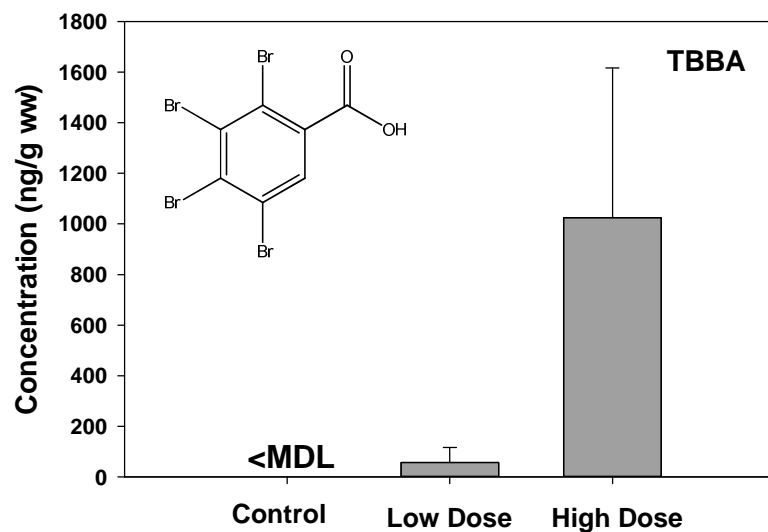


Dam Hepatic Tissue on PND 21:

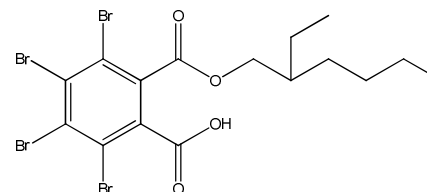
Parent
Compounds
→

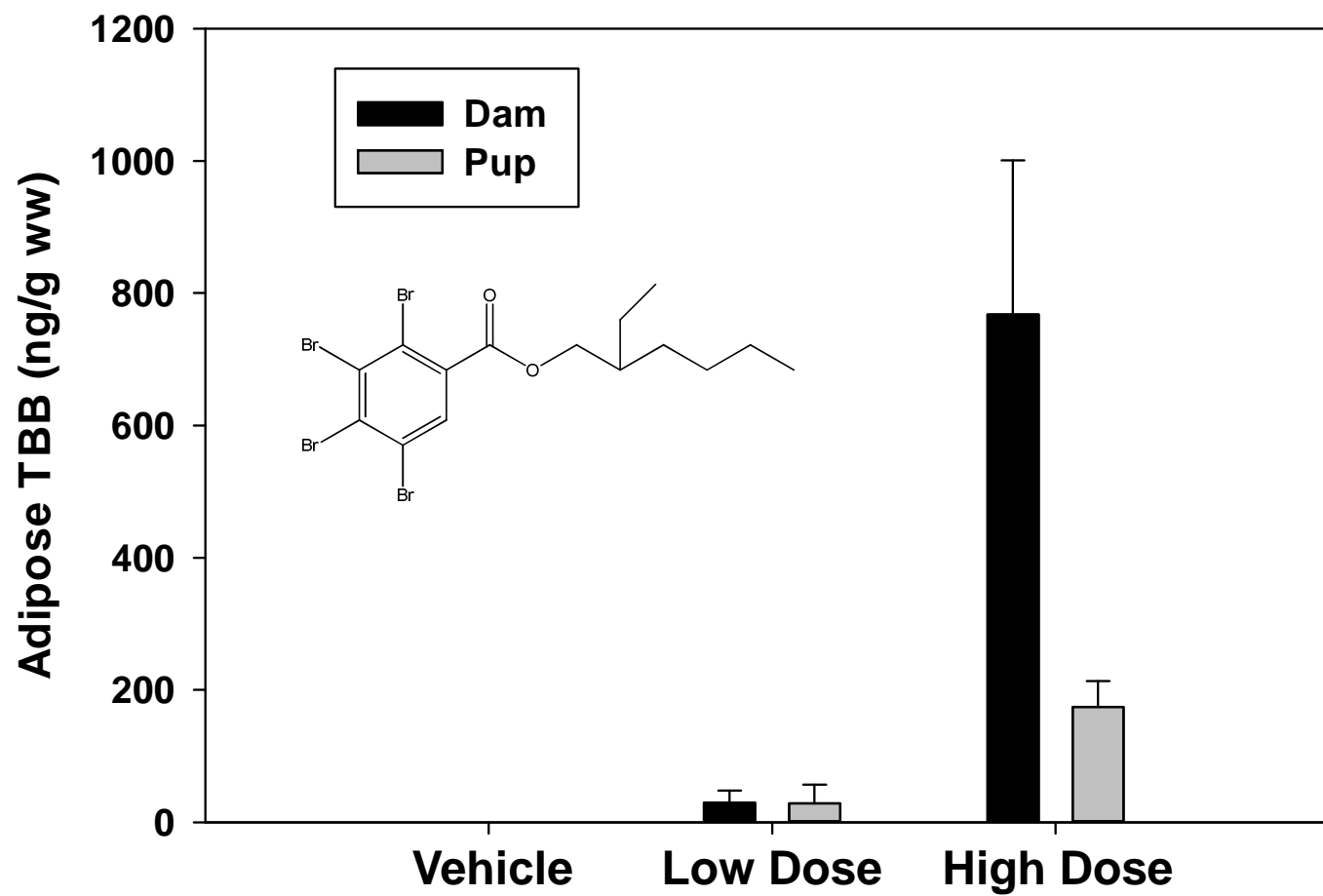
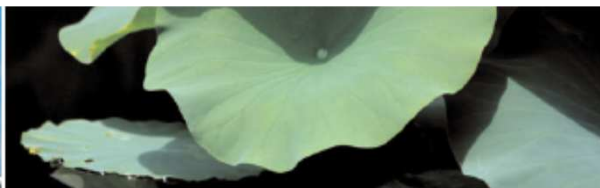


Metabolites
→



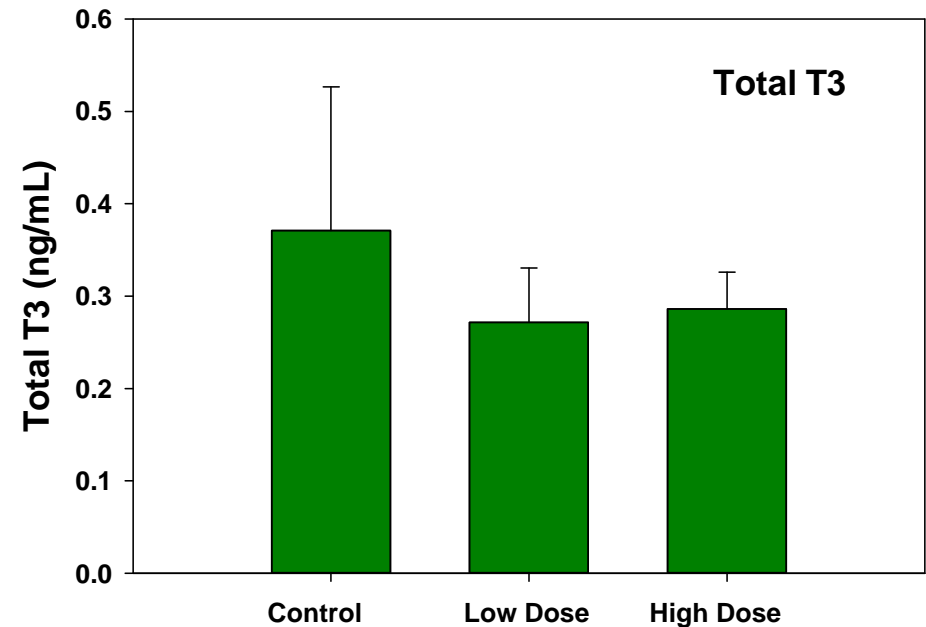
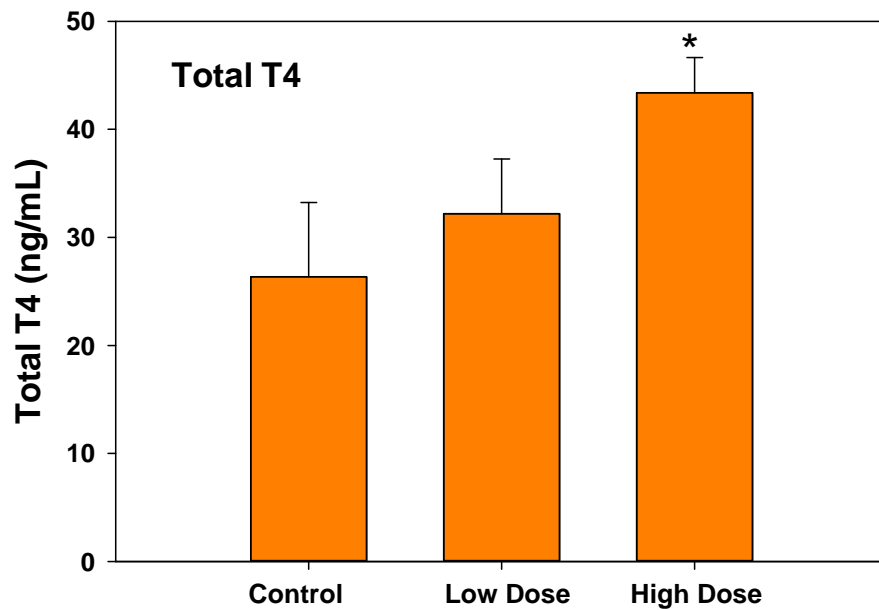
Hepatic TBMEHP <MDL







Thyroid Hormones in Dam Serum PND 21



Bars represent mean \pm 1 standard error

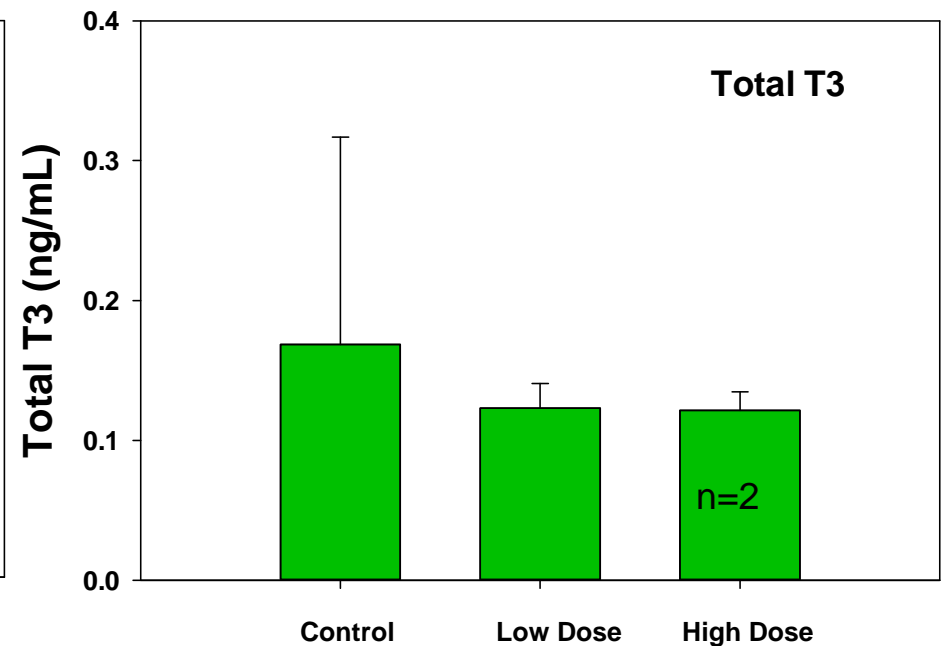
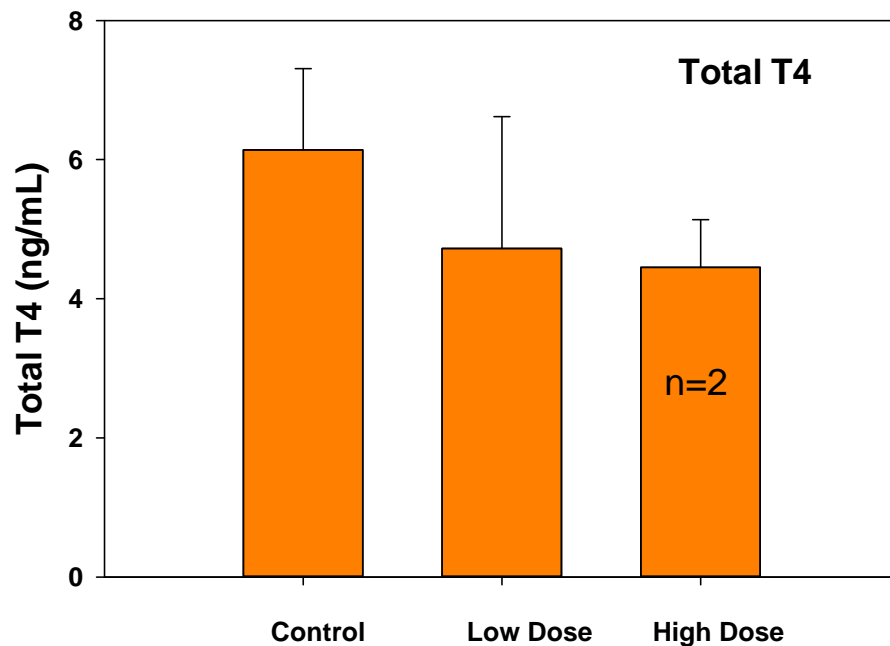
Data were analyzed using ANOVA and Dunnett's post-hoc when appropriate

* Indicates significant difference from control of $p < 0.05$

n=3 unless otherwise noted



Thyroid Hormones in Pup Serum: PND 21



Bars represent mean ± 1 standard error

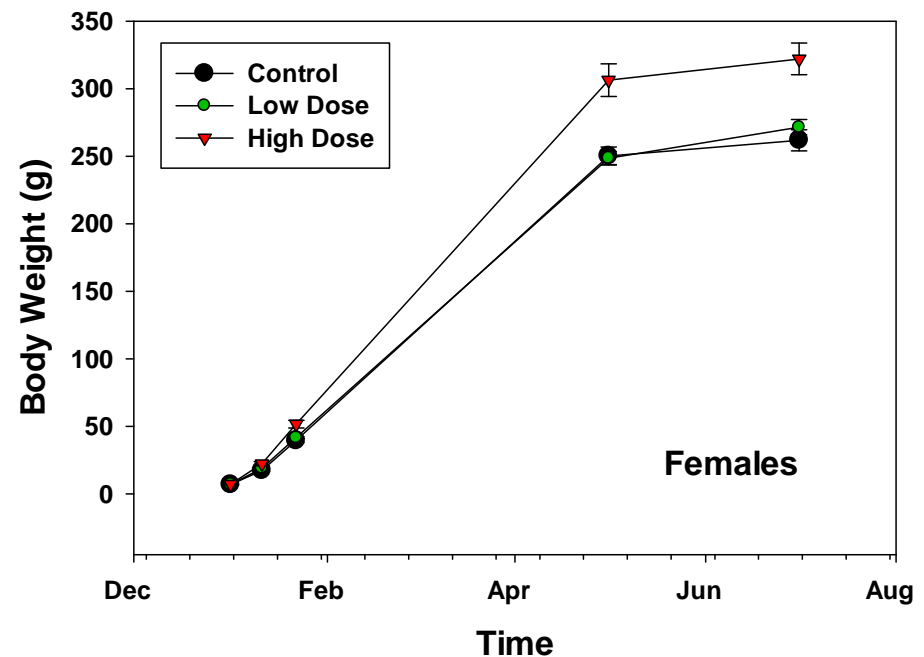
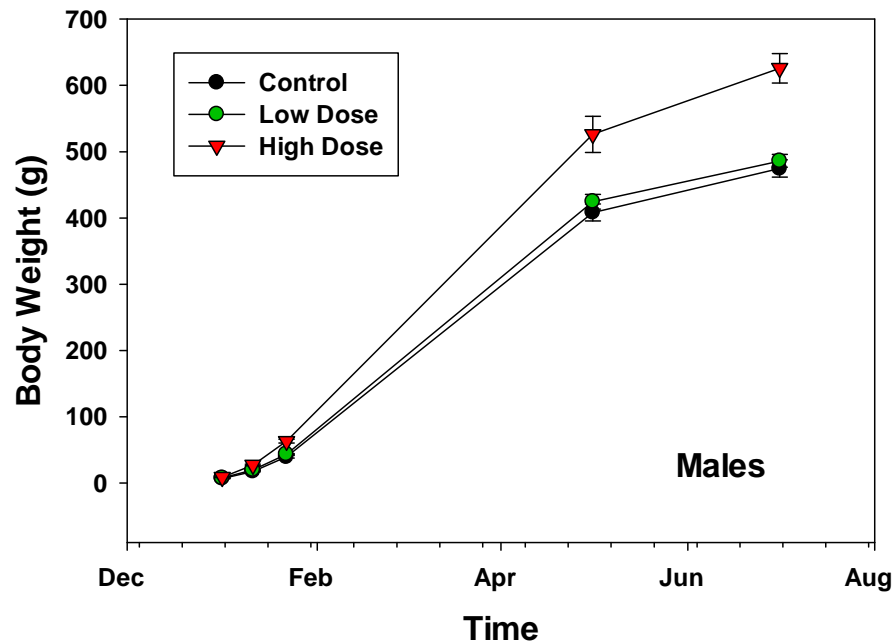
Data were analyzed using ANOVA and Dunnett's post-hoc when appropriate

* Indicates significant difference from control of $p < 0.05$

n=3 unless otherwise noted



Pup Body Weight



*Males in high dose significantly heavier than controls at all time points

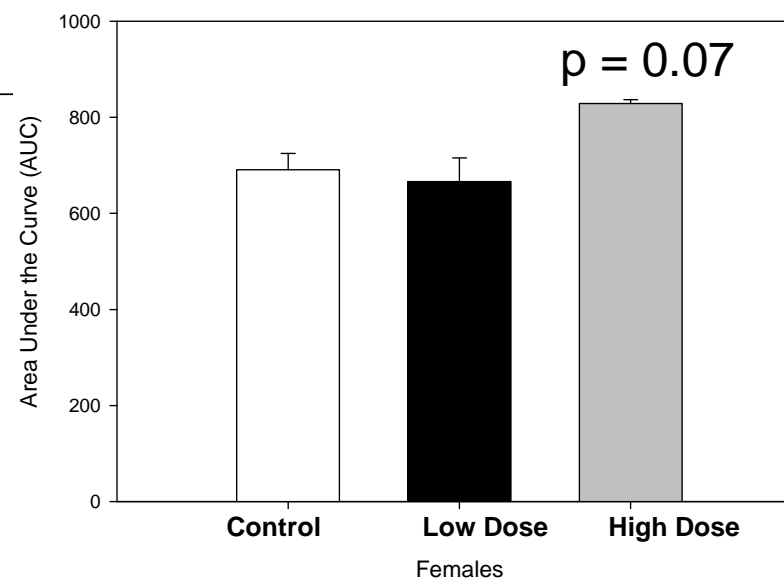
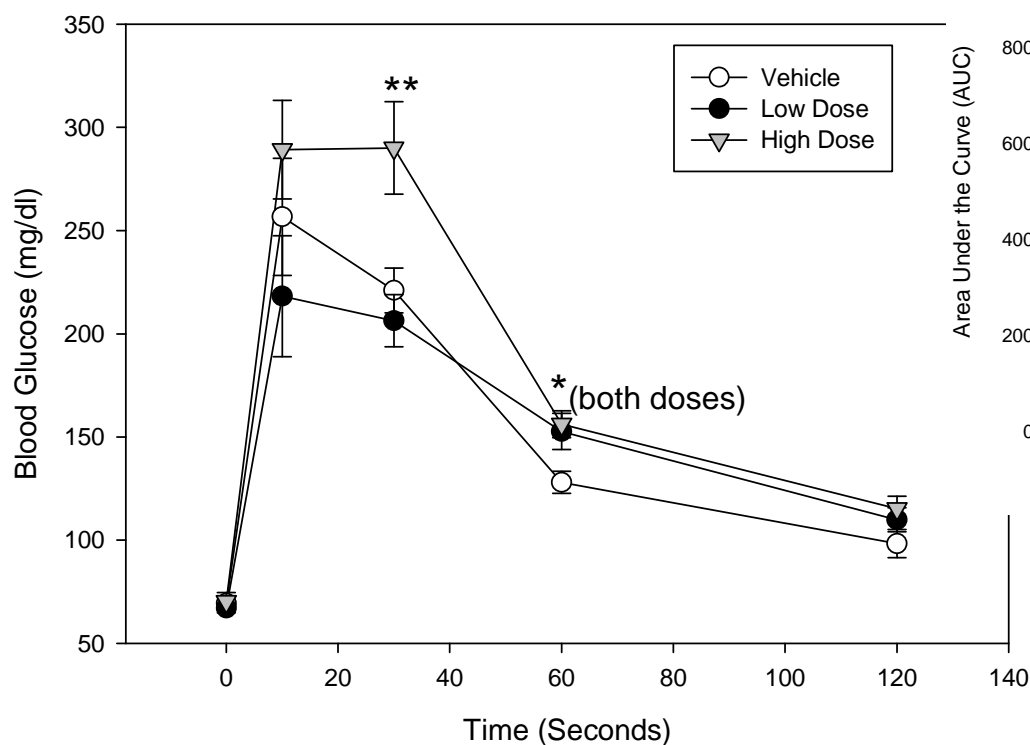
*Females in high dose significantly heavier than controls starting at PND 21

Bars represent mean \pm 1 standard error

* Indicates significant difference from control of $p < 0.05$
n=3 unless otherwise noted



Female Glucose Challenge Data (4 Months of Age)

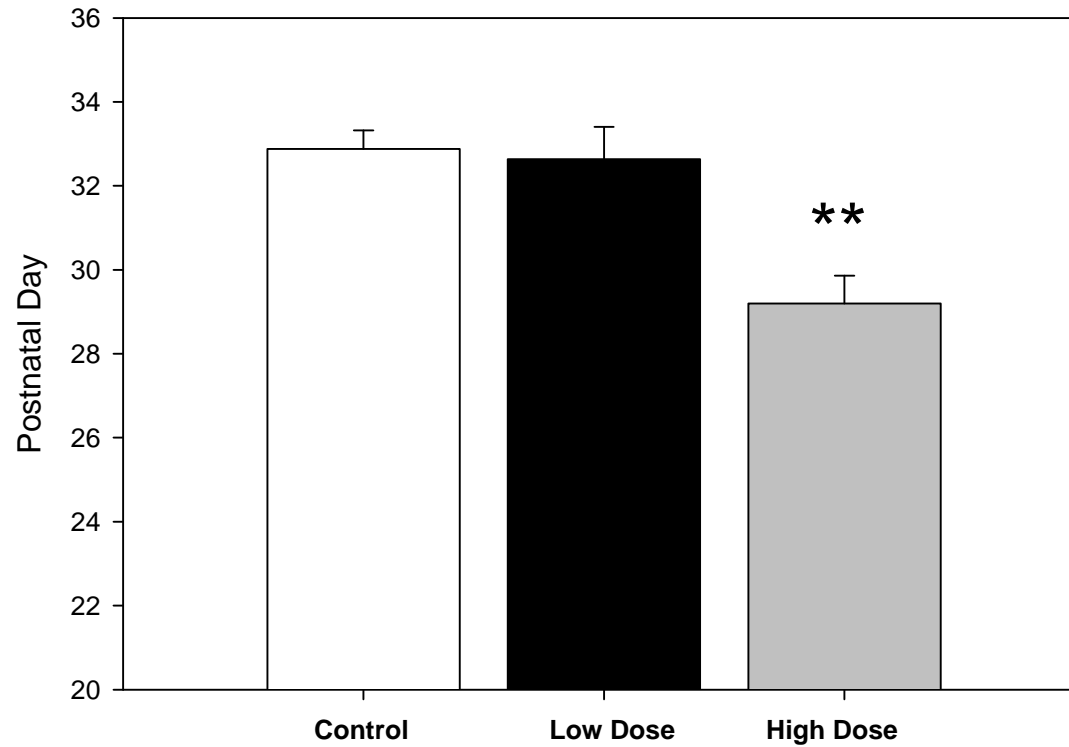


**** p<0.01; * p<0.05**



Timing of Female Pubertal Onset

Age at Vaginal Opening



** $p < 0.01$; * $p < 0.05$



Conclusions:

- Toddlers are receiving elevated exposures to FRs in house dust, likely due to hand to mouth contacts;
- Age, breast milk ingestion, SES and PBDE residues on the hands are significant predictors of serum PBDE levels in toddlers (explaining 40% of serum levels);
- Non-PBDE flame retardants are now used in furniture products and are accumulating in indoor dust at levels similar to PBDEs;
- Prenatal exposure to FM 550 in rats led to significant weight gain, changes in glucose metabolism, early puberty, and increased anxiety (based on behavioral tests; data not shown)
- What is the true NOAEL for FM 550? Should FM 550 or its components be considered an endocrine disruptor or chemical obesogen?



Acknowledgements

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- Dr. Thomas F. Webster (Boston University); Dr. Andreas Sjödén (Centers for Disease Control and Prevention); Beth Patterson, recruiters, and the study participants

